

IN THE CLAIMS

1. (Currently amended) An isolated enzyme product of plant origin designated NPPase, characterized in that its sequence contains at least one of the polypeptide fragments represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16 and SEQ ID NO: 17 and ~~shows~~ has phosphodiesterase activity.
2. (Currently amended) The enzyme product designated NPPase, according to claim 1 characterized by having a amino acid sequence deduced from a cDNA selected among SEQ ID NO: 20 or SEQ ID NO: 22.
3. (Currently amended) The enzyme product, according to claim 2 characterized in that it contains a sequence represented by SEQ ID NO: 21.
4. (Currently amended) The enzyme product, according to claim 2 characterized in that it contains a sequence represented by SEQ ID NO: 23.
5. (Currently amended) The enzyme product according to ~~any of the claims 1 to 4~~ claim 1, characterized in that catalyses the hydrolysis of nucleotide sugars in equimolar conditions to sugar-phosphate and the corresponding nucleoside monophosphate, does not hydrolyse molecules with phosphomonoester bonds, is able to hydrolyse bis-PNPP, is inhibited by molybdate, arsenate and phosphorylated molecules, its activity is not affected by reducing and chelating agents that are inhibitors of phosphodiesterases, it is sensitive to slightly basic pH and is very stable at pH between 4 and 7.5, can be glycosylated, which makes it resistant to ionic detergents of the SDS type and to the action of proteases, and recognizes, in addition to nucleotide sugars, other small molecules that possess phosphodiester and phosphosulphate bonds.
6. (Currently amended) The enzyme product as claimed in ~~any of the claims 1 to 5~~ claim 1 characterized in that it does not hydrolyse, among others, G1P, G6P, AMP, 3-phosphoglycerate, AMPc, nor nucleic acids.
7. (Currently amended) The enzyme product as claimed in ~~either one of the claims claim 1 and 6~~, characterized in that it does not require as effectors, among other divalent cations, magnesium, manganese or cobalt.
8. (Currently amended) The enzyme product as claimed in ~~any one of the claims 1 to 7~~ claim 1, characterized in that it is inhibited by orthophosphate, inorganic pyrophosphate, and phosphate esters.
9. (Currently amended) The enzyme product as claimed in ~~any one of the claims 1 to 8~~ claim 1, characterized in that its activity is not affected by, ~~among others~~, β -mercaptoethanol, EDTA, reduced cysteine or ascorbate.

10. (Currently amended) The enzyme product as claimed in any one of the claims 1 to 9 claim 1, characterized in that it is resistant to, ~~among others~~, Proteinase K or Pronase.

11. (Currently amended) The enzyme product as claimed in any one of the claims 1 to 10 claim 1, characterized in that it recognizes as a substrates, ~~among others~~, a compound selected from the group consisting of ADPG, UDPG, GDP-glucose, ADP-mannose, APS, PAPS or bis-PNPP, the preferred substrate being ADPG.

12. (Currently amended) The enzyme product as claimed in any one of the claims 1 to 11 claim 1, characterized in that it is resistant to a temperature of 65°C for 30 minutes, and in that it has an apparent molecular weight determined by gel filtration around 70 and 270 kDa for the monomeric and homopolymeric isoform respectively, ~~as well as displaying a~~ and displays a K_{eq} of the reaction of 110, its G' being -2.9 kcal/mol, and its K_m for ADPG being 0.5 mMolar.

13. (Currently amended) The enzyme product as claimed in any one of the claims 1 to 12 claim 1, characterized in that it ~~was~~ is isolated from any plant species.

14. (Currently amended) A method of for obtaining an enzyme product of plant origin with nucleotide sugar pyrophosphatase/phospho-diesterase activity (NPPase) in its soluble isoform, having an amino acid sequence that contains at least one of the polypeptide fragments represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16 and SEQ ID NO: 17, characterized comprising the steps of: extracting an extract from in that the material of plant origin is submitted to extraction of the using a buffer, said extract comprising a protein fraction, by a buffer, filtration filtering of the extract, followed by a method of purification purifying the extract by successive centrifugations and precipitations, with adjustments both of the pH and of the ionic strength of the medium, preferably including heating of the protein above 60°C and cooling in ice, and purification by gel filtration, isoelectric focusing, denaturing-gel electrophoresis, or other equivalent means of purification of proteins extracted from plant tissues.

15. (Currently amended) The method as claimed in claim 14 comprising the following steps: (1) homogenization of the plant tissue with an extraction buffer, type Mes 50 mM pH 6, EDTA 1 mM, DTT 2 mM, (2) filtration, (3) ultracentrifugation at 100 000 g, (4) precipitation of the proteins of the supernatant in ammonium sulphate, (5) resuspension of the precipitate in buffer of pH 4.2, (6) heating for at least 15 minutes at a temperature between 60 and 65°C, followed by cooling in ice, (7) centrifugation at 30 000 g, (8) concentration of the protein of the supernatant by precipitation in ammonium sulphate and resuspension at pH 6, and (9) purification by gel-filtration chromatography, isoelectric focusing and denaturing-gel electrophoresis.

16. (Previously presented) Primers represented by SEQ ID NO: 18, SEQ ID NO: 19 or

SEQ ID NO: 24.

17. (Currently amended) Use of ~~a primers~~ primer represented by SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 24 together with an mRNA from leaves of rice or barley for obtaining, by RT-PCR, a cDNA which, after being used as a probe on cDNA libraries of leaves of rice and barley, permits the isolation of cDNA's whose sequences are represented by SEQ ID NO: 20 and SEQ ID NO: 22, respectively.

18. (Previously presented) cDNA represented by SEQ ID NO: 20 that codes for an enzyme product with NPPase activity.

19. (Previously presented) Use of primers represented by SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 24 together with an mRNA from barley leaves for obtaining, by RT-PCR, a cDNA which, after being used as a probe on cDNA libraries of barley leaves, permits the isolation of cDNA whose sequence is represented by SEQ ID NO: 22.

20. (Previously presented) cDNA represented by SEQ ID NO: 22 that codes for an enzyme product with NPPase activity.

21. (Currently amended) Use of the enzyme product of ~~claims 1 to 13~~ claim 1 in the preparation of assay devices and/or compositions for application in the determination of nucleoside diphosphate sugars.

22. (Currently amended) An assay device for the determination of nucleoside diphosphate sugars, characterized in that it includes the enzyme product of ~~claims claim 3 to 13 and 19 or 20~~ claim 1 in such a way that the determination is based on the sugar-1-phosphate released during the reaction catalysed by NPPase.

23. (Currently amended) The assay device as claimed in claim 22, characterized in that the determination is based on the glucose-1-phosphate released, which is submitted to the enzyme phosphoglucomutase to produce glucose-6-phosphate, which in its turn is submitted to a coupled reaction with NAD⁺ and NADP⁺, by the action of the enzyme glucose-6-phosphate dehydrogenase ~~to, obtaining~~ obtain 6-phosphogluconate and NADH or NADPH, ~~either of which can be determined by conventional spectrophotometric methods or methods of some other kind.~~

24. (Currently amended) An assay device for the determination of nucleoside diphosphate sugars, characterized in that it includes the enzyme product of ~~claims 1 to 13~~ claim 1, in such a way that the determination is based on the nucleoside monophosphate produced during the reaction catalysed by NPPase.

25. (Currently amended) The assay device as claimed in claim 24, characterized in that the determination is based on the nucleoside monophosphate, which is able to release orthophosphate, in addition to the corresponding base, by the action of an enzyme such as 5'-nucleotidase. ~~the orthophosphate being easily determined by~~

conventional methods, for example colorimetric methods.

26. (Currently amended) The assay device as claimed in ~~any of the claims 22 to 25~~ claim 22, characterized in that the determination is based on the release of orthophosphate ~~fact that the sugar-1-phosphate and the nucleoside-monophosphate are able to release orthophosphate~~ by the action of an enzyme such as alkaline phosphatase or 5'-nucleotidase from sugar-1-phosphate and monophosphate, ~~the orthophosphate being easily determined by conventional methods, for example colorimetric methods.~~

27. (Currently amended) Use of the enzyme product of ~~claims 1 to 13~~ claim 1 in the preparation of an assay devices device and/or ~~compositions~~ composition for application in the determination of the presence of 3'-phospho-adenosine 5'-phosphosulphate (PAPS) and adenosine 5'phosphosulphate (APS).

28. (Currently amended) Use of the primers primer of ~~claims~~ claim 16 and the cDNA[~~'s~~ of claims 18 or 20] represented by SEQ ID NO: 20 that codes for an enzyme product with NPPase activity in the production of transgenic plants that express or overexpress the cDNA that codes for NPPase.

29. (Currently amended) A method of for production of a transgenic plants plant that ~~express expresses or overexpress overexpresses~~ the gene that codes for NPPase, characterized in that a transformation vector is used that contains a plasmid that includes the cDNA represented by SEQ ID NO: 20 of the gene of the ~~said~~ NPPase.

30. (Currently amended) A method of for production of a transgenic plants plant that ~~express expresses or overexpress overexpresses~~ the gene that codes for NPPase, characterized in that it uses a transformation vector that contains a plasmid that includes the cDNA represented by SEQ ID NO: 22 of the gene of the ~~said~~ NPPase.

31. (Currently amended) A method of production of transgenic plants as claimed in claim 29 ~~or 30~~, characterized in that the transformation vector is Agrobacterium tumefaciens CECT 5799.

32. (Currently amended) A transgenic plants plant obtainable by the method as claimed in ~~claims~~ claim 29 to 34, characterized in that ~~they it express expresses or overexpress overexpresses~~ the enzyme product of [claims 1 to 13] plant origin designated NPPase, characterized in that its sequence contains at least one of the polypeptide fragments represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 17 and has phosphodiesterase activity and ~~have~~ has a reduced content of starch and/or of cell-wall polysaccharides and ~~are is~~ resistant to high temperatures temperature and to high salinity.

33. (Currently amended) An assay device for the determination of sulphonucleotides, characterized in that it includes the enzyme product of ~~claims 1 to 13~~ claim 1 in such a way that the determination is based on the sulphate that is released.

34. (New) The enzyme product according to claim 1 characterized in that it is inhibited by AMP, ADP, ATP, or 3-phosphoglycerate.

35. (New) The enzyme product according to claim 1 where in the substrate is ADPG.

36. (New) A method of production of transgenic plants as claimed in claim 30, characterized in that the transformation vector is *Agrobacterium tumefaciens* CECT 5799.